AWARD NUMBER: W81XWH-05-1-0401

TITLE: Identification of Biomarkers Associated with the Healing of Chronic Wounds

PRINCIPAL INVESTIGATOR: Laura E. Edsberg, Ph.D.

CONTRACTING ORGANIZATION: Daemen College
Amherst, NY 14226

REPORT DATE: September 2012

TYPE OF REPORT: Addendum to Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
1 September 2012	Addendum to Final	1 Sep 2011 – 31 Aug 2012

4. TITLE AND SUBTITLE

Identification of Biomarkers Associated with the Healing of Chronic Wounds

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-05-1-0401

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Laura E. Edsberg, Ph. D.

E-Mail: Ledsberg@daemen.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Daemen College
Amherst, NY 14226

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

It is the objective of this study to identify the biochemical differences in the burn fluid of burns with hypertrophic scarring and those without. The findings of this study are intended to facilitate the development of diagnostic tools, which could be used to evaluate the healing process and develop therapeutic treatments. A porcine burn model has been used to evaluate healing. PIXIES was used to analyze the cytokines and growth factors present in the burn wounds.

15. SUBJECT TERMS
Burn wounds, hypertrophic scarring, antibody arrays, PIXIES

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
					USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**		9	**19b. TELEPHONE NUMBER** *(include area code)*
U	U	U	UU		

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

INTRODUCTION

Burns are among the most common injuries in modern conflicts. In recent years burn patient mortality has significantly decreased due to early wound excision and more immediate skin grafting. With increased survivorship comes the risk of hypertrophic scarring, a common complication associated with healed deep burns. Hypertrophic scarring often results in serious psychological and physical effects**.** The pathophysiology of hypertrophic scar formation is not clear and it is not understood why some patients develop hypertrophic scarring while others do not. Because hypertrophic scarring is the result of a protein imbalance at the injury site, the biochemical characterization of the burn wound from the time of injury until closure in parallel with the identification of the differences in burn fluid and burn patient sera is the important first step in developing a treatment to prevent hypertrophic scarring.

This proposal will assess the biochemical profiles of healing burns and compare the profiles from burns with hypertrophic scarring versus those without, thereby testing the hypothesis that specific quantifiable biochemical differences in the sera and burn fluid exist between burn patients that develop hypertrophic scarring and those that do not.

BODY

STATEMENT OF WORK

Continuation

Burn Fluid and Patient Sera Biochemical Analysis as an Indicator of Aberrant Wound Repair and Hypertrophic Scarring

Phase I:

Technical Objective 1: Characterize the protein biochemistry of burn wounds.

a. Analyze wound fluid samples to determine proteins present
a. Identify trends present in burns as healing occurs

Technical Objective 2: Characterize the protein biochemistry in the sera of subjects with burn wounds.

a. Analyze sera to determine the proteins present
b. Identify trends present in subjects with burns during healing

Technical Objective 3: Assess the presence of hypertrophic scarring.

a. Burn Scar Index (Vancouver Scar Scale) parameters of scar will be assessed

b. Identify subjects with hypertrophic scarring burn wounds

Technical Objective 4: Correlate the differences between the sera and burn fluid samples during healing and identify biochemical differences between hypertrophic scarring and non-hypertrophic scarring subjects.

a. Correlate the trends in wound and sera biochemistry during healing
b. Correlate clinical outcome with biochemistry
c. Identify the differences present in sera and wound exudates in samples from subjects with hypertrophic scarring

Phase II:

Technical Objective 1: Develop a porcine model for burn wounds (second degree - superficial and deep).

a. Develop methods to reproducibly induce cutaneous thermal injuries in porcine tissue model.

b. Collect wound fluid from thermally injured swine for proteins of clinical interest, based upon those identified in Phase I of this project.

Technical Objective 2: Characterize the protein biochemistry of porcine wound fluids.

a. Analyze burn wound fluid by both ELISA and PIXIES.

b. Compare results from PIXIES with those from ELISA.

Technical Objective 3: Evaluate and validate porcine data with those obtained from Phase I studies.

a. Compare wound fluid biochemistry from thermally injured swine to that from normally-healing human wound fluid from Phase I of the study.

Phase I

Technical Objectives 1, 2, & 3:

Completion of all Phase I technical objectives has been delayed due to slow enrollment. In March 2012 a modification to the inclusion criteria for subjects was approved. This modification allows the enrollment of subjects with 2% and greater TBSA burns to be enrolled versus 5% and greater. The modified protocol and consent forms were reviewed and approved by the Daemen College Human Subjects Research Review Committee and University at Buffalo Health Sciences Institutional Review Board. The modification was reviewed by the U.S. Army Medical Research and Materiel Command (USAMRMC), Office of Research Protections (ORP), Human Research Protection Office (HRPO) and found to comply with applicable Federal, DOD,

U.S. Army, and USAMRMC human subjects protection requirements (A-13375.2b - HRPO Approval Memorandum (Proposal Log Number 05053002, Award Number W81XWH-05-1-0401, HSIRB Project# MED7020211A).

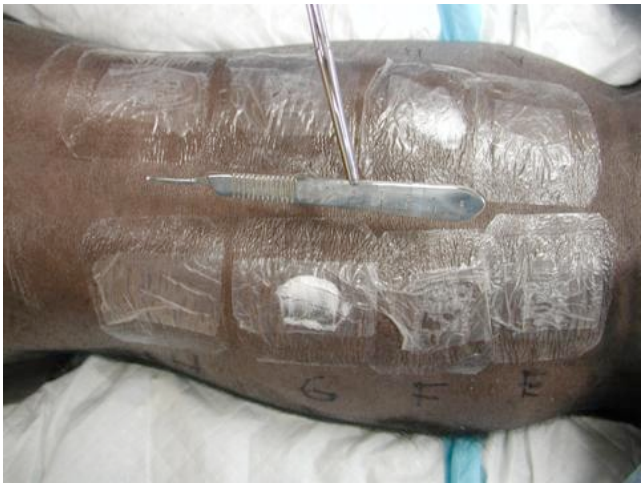
Phase II

Technical Objective 1: *Develop a porcine model for burn wounds (second degree – superficial and deep)*

a. *Develop methods to reproducibly induce cutaneous injuries in porcine tissue model*
b. *Collect wound fluid from thermally injured swine for proteins of clinical interest, based on those identified with phase I of this project*

a. Porcine second degree burns were inflicted on the paravertebral region of anesthetized, surgically prepared pigs, according to IACUC-approved protocol. Aluminum bars were heated to 90°C in a water bath, then dried off and applied to the skin for 40 seconds with all pressure due to gravity alone. These parameters were based on a review of the literature (see references). It was found that if the animal did not have a broad enough back, then the bars were not level on the skin and the burns produced were uneven in area and depth.

b. Fluids were collected from all animals, regardless of burn depth – as a model for the heterogeneity of human thermal injuries. Fluids were stored at – 80°C for subsequent protein analysis.



Deep partial-thickness thermal injury to paravertebral area of domestic Yorkshire Cross pig. Burns were covered with transparent occlusive dressings and wound fluids were collected at each dressing change - every 3 days, until termination at day 10 post-injury.

Technical Objective 2: *Characterize the protein biochemistry of porcine wound fluids*
a. *Analyze burn wound fluid by both ELISA and PIXIES*
b. *Compare results from PIXIES with those from ELISA*

a. PIXIES and ELISA analysis were performed on porcine wound fluids for the following proteins: KGF, IL-1, IL-6, IL-8, IL-12, TNF-alpha. In addition, the PIXIES platform has been

used for TGF-alpha and beta, VEGF and EGF detection in untreated biological samples. Spiked biological samples were also assessed to establish correlations. Only KGF, IL-1, IL-6, IL-8 and IL-12were found in the biological specimens.
b. *Compare results from PIXIES with those from ELISA*

Correlation analysis (PIXIES detected concentration vs. ELSIA detected concentration) for the aforementioned protein targets (0-200 pg/mL) in spiked biological porcine specimens is shown below.

Target Protein Plot (pg/mL)	**Slope of Correlation Plot**	**Intercept of Correlation**
KGF	1.02	0.002
IL-1	0.96	-0.02
IL-6	1.03	-0.002
IL-8	1.002	0.001
IL-12	1.05	-0.005
TNF-alpha	1.00	0.015
TGF-alpha	0.94	0.001
TGF-beta	1.03	0.001
VEGF	1.04	-0.15
EGF	1.05	0.002

These results reveal the suitability of the PIXIES platform for determining and quantifying the aforementioned protein targets.

Technical Objective 3: *Evaluate and validate porcine data with those obtained from Phase I studies*
Compare wound fluid biochemistry from thermally injured swine to that of normally-healing human wound fluid from Phase I of the study

Porcine wound fluids await additional analyses, which are part of Phase I. PIXIES analysis has been performed on wound fluids for the proteins indicated above. Additional analysis will be run with samples from human subjects when enrollment has increased.

Key Research Accomplishments
- Development of porcine thermal injury model system (Phase II)
- Collection of porcine burn wound fluid at several time intervals post-injury (Phase II)
- Analyses of wound fluids for proteins using PIXIES (Phase II)
- Demonstrated viability of PIXIES platform for assessing wound fluid biochemistry (Phase II)

Reportable Outcomes
- Abstract/poster presentation of *in vivo* burn model to American Head and Neck Society meeting, Toronto, July 2012
- NIH RO1 grant application utilizing *in vivo* burn model system submitted 03/12
- AO foundation grant application utilizing porcine thermal injury model submitted 06/12

Several related peer-reviewed journal publications have derived from DOD sponsorship:

- Z. Tao, E.C. Tehan, R.M. Bukowski, Y. Tang, E.L. Shughart, W.G. Holthoff, A.N. Cartwright, A.H. Titus and F.V. Bright, "Templated Xerogels as Platforms for Biomolecule-less Biomolecule Sensors," Anal. Chim. Acta 2006, 564, 59-65.
- L.T. Tan, W.G. Holthoff, J.M. Steves and F.V. Bright,"Probe-dependent Microenvironments within Biodegradable Films Formed from Poly (L-lactic acid) and Pluronic 104," Appl. Spectrosc. 2010, 64, 359-364.
- P.J.R. Roche, M.C-K Cheung, K.Y. Yung, A.G. Kirk, V.P. Chodavarpu and F.V. Bright, "Application of Gold Quenching of Luminescence to Improve Oxygen Sensing using a Ruthenium (4,7-diphenyl-1,10-phenanthroline)3 Cl2:TEOS Thin Film," Sens. Actu.: B Chem. 2010, 147, 581-586.
- S.A. Burns, R. Hard, W.L. Hicks Jr., F.V. Bright, D. Cohan, L. Sigurdson and J.A. Gardella Jr., "Determining the Protein Drug Release Characteristics and Cell Adhesion to a PLLA or PLGA Biodegradable Polymer Membrane," J. Biomed. Mater.Res. Part A 2010, 94A, 27-37. L. Yao, K.Y. Yung, R. Khan, V.P. Chodavarapu, and F.V. Bright, "CMOS Imaging of Pin-Printed Xerogel based Luminescent Sensor Microarrays," IEEE Sens. J. 2010, 10, 1824-1832.
- E.L. Holthoff and F.V. Bright, "Photophysics Associated with Site Selectively Templated and Tagged Xerogel Sensor Platforms," Appl. Spectrosc. 2010, 64, 714-719.
- E.L. Holthoff and F.V. Bright, "Dynamics within a Site Selectively Templated and Tagged Xerogel Sensor Platforms," Appl. Spectrosc. 2010, 64, 1073-1077.
- L. Yao, L.; K.Y. Yung, V.P. Chodavarapu and F.V. Bright, "CMOS Imaging of Temperature Effects on Pin-Printed Xerogel Sensor Micorarrays," IEEE Trans. Biomed. Circuit. 2011, 5, 189-196.
- D.S. Daivasagaya; L. Yao, K.Y. Yung, M. Hajj-Hassan, M.C. Cheung, V.P. Chodavarapu,* F.V. Bright,* "Contact CMOS Imaging of Gaseous Oxygen Sensor Array," *Sens. Actuat. B: Chem.* **2011**, *157*, 408-416.
- K.Y. Yung, H. Xu, K. Liu, G.J. Martinez, F.V. Bright*, M.R. Detty and A.N. Cartwright, "Hybrid Oxygen-Responsive Reflective Bragg Grating Platforms," *Anal. Chem.* **2012**, *84*, 1402-1407.
- M.C. Chung, K.Y. Yung, H. Xu, N.D. Kraut, K. Liu, V.P. Chodavarapu, A.N. Cartwright and F.V. Bright*,Porous Nanostructured Encapsulation and Immobilization Materials for Optical Biosensors," *J. Selec. Top. Quant. Electron.* **2012**, *18*, 1147-1159.
- N.D. Kraut, J.D. Brattlie, R.E. Deuro, M.M. McGoorty and F.V. Bright*, "High-Throughput Screening System for Creating and Assessing Surface-Modified Porous Silicon," *Appl. Spectrosc.* **2012** in press.
- Z. Zhan, B. Zhou, Z. Fu, F.V. Bright, A.N. Cartwright and A.H. Titus*, "Filterless Optical Oxygen Sensor Based on a CMOS Buried Double Junction Photodiode," *Sens. Actu : Chem. B* **2012** in press.

Conclusion

- All but one of Phase II objectives have been met.
- Technical objective 3 of Phase II is delayed by the enrollment issues in Phase I and will coordinate with Phase I for comparison of porcine and human wound fluid biochemistry when samples are available.

References

- Hoekstra, MJ., Hupkens, P., Dutrieux, RP., Bosch, MMC., Brans, TA., Kreis, RW. (1993). A comparative burn wound model in the New Yorkshire pig for the histopathological evaluation of local therapeutic regimens: silver sulfadiazine cream as a standard. Br. J. Plast. Surg. 46:585-589.

- Singer, AJ., Berruti, L., Thode, HC., McClain, SA. (1999). Octylcyanoacrylate for the treatment of partial-thickness burns in swine: a randomized, controlled experiment. Acad. Emerg. Med. 6:688-692.
- Singer, AJ., Berruti, L., Thode, Jr., HC., McClain, SA.. (2000). Standardized burn model using a multiparametric histologic analysis of burn depth. Acad. Emerg. Med. 7(1):1-6.

- Cuttle, L., Kempf, M., Phillips, GE., Mill, J., Hayes, MT., Fraser, JF., Wang, X-Q., Kimble, RM. (2006). A porcine deep dermal partial thickness burn model with hypertrophic scarring. Burns 32:806-820.

- Singer, AJ., Huang, SS., Huang, JS., McClain, SA., Romanov, A., Rooney, J., Zimmerman, T. (2009). A novel TGF-beta antagonist speeds reepithelialization and reduces scarring of partial thickness porcine burns. J. Burn Care and Research 30:329-334.